

WHAT IS CLAIMED IS:

1. An isolated or recombinant nucleic acid comprising
a nucleic acid sequence having at least 96% sequence identity to SEQ ID
NO:1 over a region of at least about 100 residues, or
5 a nucleic acid sequence having at least 95% sequence identity to SEQ ID
NO:3 over a region of at least about 100 residues,
wherein the nucleic acid encodes at least one polypeptide having a xylose
isomerase activity, and the sequence identities are determined by analysis with a sequence
comparison algorithm or by a visual inspection.
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2. The isolated or recombinant nucleic acid of claim 1, wherein the
nucleic acid comprises
a nucleic acid sequence having at least 96% sequence identity to SEQ ID
NO:1 over a region of at least about 200 residues, or
15 a nucleic acid sequence having at least 95% sequence identity to SEQ ID
NO:3 over a region of at least about 200 residues.
3. The isolated or recombinant nucleic acid of claim 2, wherein the
nucleic acid comprises
20 a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1
over a region of at least about 300 residues, or
a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3
over a region of at least about 300 residues.
- 25 4. The isolated or recombinant nucleic acid of claim 3, wherein the
nucleic acid comprises
a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1
over a region of at least about 400 residues, or
a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3
30 over a region of at least about 400 residues.
5. The isolated or recombinant nucleic acid of claim 4, wherein the
nucleic acid comprises

a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 500 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 500 residues.

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6. The isolated or recombinant nucleic acid of claim 5, wherein the nucleic acid comprises

a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 600 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 600 residues.

7. The isolated or recombinant nucleic acid of claim 6, wherein the nucleic acid comprises

15 a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 700 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 700 residues.

20 8. The isolated or recombinant nucleic acid of claim 7, wherein the nucleic acid comprises

a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 800 residues, or

25 a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 800 residues.

9. The isolated or recombinant nucleic acid of claim 8, wherein the nucleic acid comprises

30 a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 900 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 900 residues.

10. The isolated or recombinant nucleic acid of claim 9, wherein the nucleic acid comprises

a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 1000 residues, or

5 a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 1000 residues.

11. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid comprises

10 a nucleic acid sequence having at least 97% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues.

12. The isolated or recombinant nucleic acid of claim 11, wherein the nucleic acid comprises

a nucleic acid sequence having at least 98% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

20 a nucleic acid sequence having at least 97% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues.

13. The isolated or recombinant nucleic acid of claim 12, wherein the nucleic acid sequence comprises

25 a nucleic acid sequence having at least 99% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 98% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues.

14. The isolated or recombinant nucleic acid of claim 13, wherein the nucleic acid comprises

30 a nucleic acid sequence having at least 99% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues.

15. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence comprises

a nucleic acid having a sequence as set forth in SEQ ID NO:1, or
a nucleic acid having a sequence as set forth in SEQ ID NO:3.

16. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence encodes a polypeptide comprising a sequence as set forth in SEQ ID NO:2, or, a sequence as set forth in SEQ ID NO:4, or subsequences thereof.

17. The isolated or recombinant nucleic acid of claim 1, wherein the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default.

18. The isolated or recombinant nucleic acid of claim 1, wherein the xylose isomerase activity comprises isomerization of xylose to xylulose.

19. The isolated or recombinant nucleic acid of claim 1, wherein the xylose isomerase activity comprises isomerization of glucose to fructose.

20. The isolated or recombinant nucleic acid of claim 19, wherein the xylose isomerase activity comprises isomerization of D-glucose to D-fructose.

21. The isolated or recombinant nucleic acid of claim 1, wherein the xylose isomerase activity comprises catalysis of the conversion of D-xylose to an equilibrium mixture of D-xylulose and D-xylose.

22. The isolated or recombinant nucleic acid of claim 1, wherein the xylose isomerase activity comprises isomerization of α -D-glucopyranose to α -D-fructofuranose.

23. The isolated or recombinant nucleic acid of claim 1, wherein the xylose isomerase activity comprises isomerization of β -D-glucopyranose to β -D-fructopyranose.

24. The isolated or recombinant nucleic acid of claim 1, wherein the xylose isomerase activity is thermostable.

25. The isolated or recombinant nucleic acid of claim 24, wherein the polypeptide retains a xylose isomerase activity under conditions comprising a temperature range of between about 60°C to about 120°C, or, between about 60°C to about 95°C.

26. The isolated or recombinant nucleic acid of claim 24, wherein the polypeptide retains a xylose isomerase activity under conditions comprising a temperature range of between about 95°C to about 105°C, or, between about 105°C to about 120°C.

27. The isolated or recombinant nucleic acid of claim 1, wherein the xylose isomerase activity is thermotolerant.

28. The isolated or recombinant nucleic acid of claim 27, wherein the polypeptide retains a xylose isomerase activity after exposure to conditions comprising a temperature range of between about 95°C to about 135°C, or, between about 95°C to about 105°C.

29. The isolated or recombinant nucleic acid of claim 27, wherein the polypeptide retains a xylose isomerase activity after exposure to conditions comprising a temperature range of between about 105°C to about 120°C, or, between about 120°C to about 135°C.

30. An isolated or recombinant nucleic acid, wherein the nucleic acid comprises a sequence that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a sequence as set forth in SEQ ID NO:3, wherein the nucleic acid encodes a polypeptide having a xylose isomerase activity.

31. The isolated or recombinant nucleic acid of claim 30 wherein the nucleic acid is at least about 100 residues in length.

32. The isolated or recombinant nucleic acid of claim 31 wherein the nucleic acid is at least about 200 residues in length.

33. The isolated or recombinant nucleic acid of claim 32, wherein the nucleic acid is at least about 300 residues in length.

34. The isolated or recombinant nucleic acid of claim 33, wherein the nucleic acid is at least about 400 residues in length.

35. The isolated or recombinant nucleic acid of claim 34, wherein the nucleic acid is at least about 500, 600, 700, 800, 900, 1000 residues in length or the full length of the gene or transcript.

36. The isolated or recombinant nucleic acid of claim 30, wherein the stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

37. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide comprising a xylose isomerase activity, wherein the probe comprises at least 10 consecutive bases of a sequence comprising:

a sequence as set forth in SEQ ID NO:1, or

a sequence as set forth in SEQ ID NO:3,

wherein the probe identifies the nucleic acid by binding or hybridization.

38. The nucleic acid probe of claim 37, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 consecutive bases of a sequence comprising

a sequence as set forth in SEQ ID NO:1, or

a sequence as set forth in SEQ ID NO:3.

39. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a xylose isomerase activity, wherein the probe comprises a nucleic acid comprising

a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,

5 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

40. The nucleic acid probe of claim 39, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 consecutive bases of a nucleic acid sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof.

41. The nucleic acid probe of claim 39, wherein the probe comprises a nucleic acid sequence having at least 97% sequence identity to a region of at least about 100 residues of a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

42. The nucleic acid probe of claim 41, wherein the probe comprises a nucleic acid sequence having at least 98% sequence identity to a region of at least about 100 residues of a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

43. The nucleic acid probe of claim 42, wherein the probe comprises a nucleic acid sequence having at least 99% sequence identity to a region of at least about 100 residues of a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

44. An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a xylose isomerase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

45. The amplification primer pair of claim 44, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence.

5 46. A method of amplifying a nucleic acid encoding a polypeptide having xylose isomerase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

47. An expression cassette comprising a nucleic acid comprising:
(i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or
a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,
15 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,
(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

20 48. The expression cassette of claim 47, wherein the nucleic acid is operably linked to a plant promoter.

25 49. The expression cassette of claim 48 further comprising a plant expression vector.

50. The expression cassette of claim 49, wherein the plant expression vector comprises a plant virus.

30 51. The expression cassette of claim 48, wherein the plant promoter comprises a potato promoter, a rice promoter, a corn promoter, a wheat promoter or a barley promoter.

52. The expression cassette of claim 47, wherein the promoter comprises a promoter derived from T-DNA of *Agrobacterium tumefaciens*.

53. The expression cassette of claim 47, wherein the promoter is a constitutive promoter.

54. The expression cassette of claim 47, wherein the promoter is an inducible promoter.

55. The expression cassette of claim 47, wherein the promoter is a tissue-specific promoter.

56. The expression cassette of claim 55, wherein the tissue-specific promoter is a seed-specific, a leaf-specific, a root-specific, a stem-specific or an abscission-induced promoter.

57. A vector comprising a nucleic acid comprising
(i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or
a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,
wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,
(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

58. A cloning vehicle comprising a vector as set forth in claim 57, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

59. The cloning vehicle of claim 58, wherein the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector.

60. The cloning vehicle of claim 58, comprising a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

5 61. A transformed cell comprising a vector, wherein the vector comprises
(i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or
a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,
10 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,
(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

15 62. A transformed cell comprising
(i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or
a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,
20 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,
(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

25 63. The transformed cell of claim 61 or claim 62, wherein the cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.

30 64. The transformed cell of claim 63, wherein the plant cell is a potato, rice, corn, wheat, tobacco, rapeseed, grass, soybean or barley cell.

65. A transgenic non-human animal comprising

(i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,

5 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

10 66. The transgenic non-human animal of claim 76, wherein the animal is a mouse.

67. A transgenic plant comprising

15 (i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues,

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,

20 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

25 68. The transgenic plant of claim 67, wherein the plant is a corn plant, a potato plant, a grass, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant or a tobacco plant.

69. A method of making a transgenic plant comprising the following steps:

30 (a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 41, thereby producing a transformed plant cell;

(b) producing a transgenic plant from the transformed cell.

70. A transgenic seed comprising

(i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

71. The transgenic seed of claim 70, wherein the seed is a starch granule or grain, corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a peanut or a tobacco plant seed.

72. An antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to

(i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

73. The antisense oligonucleotide of claim 72, wherein the antisense oligonucleotide is between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 bases in length.

74. A method of inhibiting the translation of a xylose isomerase message in a cell comprising administering to the cell or expressing in the cell an antisense

oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid comprising

(i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

5 a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid
10 comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

75. An isolated or recombinant polypeptide comprising

(a) a polypeptide comprising

15 an amino acid sequence having at least 96% identity to SEQ ID NO:2 over a region of at least about 100 residues, or

an amino acid sequence having at least 95% identity to SEQ ID NO:4 over a region of at least about 100 residues, or

(b) a polypeptide encoded by a nucleic acid comprising

20 (i) a nucleic acid sequence having at least 96% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues,

25 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 or subsequences thereof.

30 76. The isolated or recombinant polypeptide of claim 75, wherein the polypeptide comprises a xylose isomerase activity.

77. The isolated or recombinant polypeptide of claim 75, wherein the polypeptide comprises

an amino acid sequence having at least 96% identity to SEQ ID NO:2 over a region of at least about 200 residues, or

an amino acid sequence having at least 95% identity to SEQ ID NO:4 over a region of at least about 200 residues.

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78. The isolated or recombinant polypeptide of claim 77, wherein the polypeptide comprises

an amino acid sequence having at least 96% identity to SEQ ID NO:2 over a region of at least about 300 residues, or

an amino acid sequence having at least 95% identity to SEQ ID NO:4 over a region of at least about 300 residues.

79. The isolated or recombinant polypeptide of claim 78, wherein the polypeptide comprises

15 an amino acid sequence having at least 96% identity to SEQ ID NO:2 over a region of at least about 400 residues, or

an amino acid sequence having at least 95% identity to SEQ ID NO:4 over a region of at least about 400 residues.

20 80. The isolated or recombinant polypeptide of claim 79, wherein the polypeptide comprises an amino acid sequence having at least 96% identity to SEQ ID NO:2, or an amino acid sequence having at least 95% identity to SEQ ID NO:4.

25 81. The isolated or recombinant polypeptide of claim 75, wherein the polypeptide comprises

an amino acid sequence having at least 97% identity to SEQ ID NO:2 over a region of at least about 100 residues, or

an amino acid sequence having at least 96% identity to SEQ ID NO:4 over a region of at least about 100 residues.

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82. The isolated or recombinant polypeptide of claim 81, wherein the polypeptide comprises

an amino acid sequence having at least 98% identity to SEQ ID NO:2 over a region of at least about 100 residues, or

an amino acid sequence having at least 97% identity to SEQ ID NO:4 over a region of at least about 100 residues.

83. The isolated or recombinant polypeptide of claim 82, wherein the polypeptide comprises

an amino acid sequence having at least 99% identity to SEQ ID NO:2 over a region of at least about 100 residues, or

an amino acid sequence having at least 98% identity to SEQ ID NO:4 over a region of at least about 100 residues.

84. The isolated or recombinant polypeptide of claim 83, wherein the polypeptide comprises an amino acid sequence having at least 99% identity to SEQ ID NO:4 over a region of at least about 100 residues.

85. The isolated or recombinant polypeptide of claim 84, wherein the polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:2, or a subsequence thereof, an amino acid sequence as set forth in SEQ ID NO:4, or a subsequence thereof.

86. The isolated or recombinant polypeptide of claim 76, wherein the xylose isomerase activity comprises isomerization of xylose to xylulose.

87. The isolated or recombinant polypeptide of claim 76, wherein the xylose isomerase activity comprises isomerization of glucose to fructose.

88. The isolated or recombinant polypeptide of claim 87, wherein the xylose isomerase activity comprises isomerization of D-glucose to D-fructose.

89. The isolated or recombinant polypeptide of claim 76, wherein the xylose isomerase activity comprises catalysis of the conversion of D-xylose to an equilibrium mixture of D-xylulose and D-xylose.

90. The isolated or recombinant polypeptide of claim 76, wherein the xylose isomerase activity comprises isomerization of α -D-glucopyranose to α -D-fructofuranose.

5 91. The isolated or recombinant polypeptide of claim 76, wherein the xylose isomerase activity comprises isomerization of β -D-glucopyranose to β -D-fructopyranose.

10 92. The isolated or recombinant polypeptide of claim 76, wherein the xylose isomerase activity is thermostable.

15 93. The isolated or recombinant polypeptide of claim 92, wherein the polypeptide retains a xylose isomerase activity under conditions comprising a temperature range of between about 60°C to about 120°C, or, between about 60°C to about 95°C.

94. The isolated or recombinant polypeptide of claim 92, wherein the polypeptide retains a xylose isomerase activity under conditions comprising a temperature range of between about 95°C to about 105°C, or, between about 105°C to about 120°C.

20 95. The isolated or recombinant polypeptide of claim 76, wherein the xylose isomerase activity is thermotolerant.

25 96. The isolated or recombinant polypeptide of claim 95, wherein the polypeptide retains a xylose isomerase activity under conditions comprising a temperature range of between about 95°C to about 135°C, or, between about 95°C to about 105°C.

30 97. The isolated or recombinant polypeptide of claim 95, wherein the polypeptide retains a xylose isomerase activity under conditions comprising a temperature range of between about 105°C to about 120°C, or, between about 120°C to about 135°C.

98. An isolated or recombinant polypeptide comprising the polypeptide as set forth in claim 75 and lacking a signal sequence.

99. An isolated or recombinant signal sequence peptide comprising a sequence as set forth in the amino terminal 20 to 30 residues of SEQ ID NO:2 or SEQ ID NO:4.

5 100. The isolated or recombinant polypeptide of claim 76, wherein the xylose isomerase activity comprises a specific activity at about 95°C in the range from about 100 to about 1000 units per milligram of protein, or, a specific activity from about 500 to about 750 units per milligram of protein, or, a specific activity at 95°C in the range from about 500 to about 1200 units per milligram of protein, or, a specific activity at 95°C in the
10 range from about 750 to about 1000 units per milligram of protein.

101. The isolated or recombinant polypeptide of claim 75, wherein the polypeptide comprises at least one glycosylation site.

15 102. The isolated or recombinant polypeptide of claim 101, wherein glycosylation is an N-linked glycosylation.

103. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide is glycosylated after being expressed in a *P. pastoris* or a *S. pombe*.

20 104. The isolated or recombinant polypeptide of claim 75, wherein the polypeptide retains a xylose isomerase activity under conditions comprising about pH 4.0, 4.5, 5.0, 5.5, 6.0 or 6.5.

25 105. The isolated or recombinant polypeptide of claim 75, wherein the polypeptide retains a xylose isomerase activity under conditions comprising about pH 8.0, 8.5, 9.0, 9.5, 10, 10.5 or 11.

30 106. A protein preparation comprising a polypeptide as set forth in claim 75, wherein the protein preparation comprises a liquid, a solid or a gel.

107. A homodimer comprising a polypeptide as set forth in claim 75.

108. A heterodimer comprising a polypeptide as set forth in claim 75 and a second domain.

109. The heterodimer of claim 108, wherein the second domain is a polypeptide and the heterodimer is a fusion protein.

110. The heterodimer of claim 108, wherein the second domain is an epitope.

111. The heterodimer of claim 108, wherein the second domain is a tag.

112. An immobilized polypeptide having a xylose isomerase activity, wherein the polypeptide comprises a sequence as set forth in claim 75 or claim 108.

113. The immobilized polypeptide of claim 112, wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.

114. An array comprising an immobilized polypeptide as set forth in claim 75 or claim 108.

115. An array comprising an immobilized nucleic acid as set forth in claim 1 or claim 30.

116. An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 75 or to a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30.

117. The isolated or recombinant antibody of claim 116, wherein the antibody is a monoclonal or a polyclonal antibody.

118. A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 75 or to a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30.

119. A food supplement for an animal comprising a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30.

5 120. The food supplement of claim 119, wherein the polypeptide is glycosylated.

121. The food supplement of claim 119 comprising a glucose or a starch.

10 122. An edible enzyme delivery matrix comprising a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30, wherein the polypeptide comprises a xylose isomerase activity.

15 123. The edible enzyme delivery matrix of claim 122 comprising a glucose or a starch.

124. The edible enzyme delivery matrix of claim 122, wherein the delivery matrix comprises a pellet.

20 125. The edible enzyme delivery matrix of claim 122, wherein the polypeptide is glycosylated.

25 126. The edible enzyme delivery matrix of claim 122, wherein the xylose isomerase activity is thermotolerant or thermostable.

127. A method of isolating or identifying a polypeptide with a xylose isomerase activity comprising the steps of:

(a) providing an antibody as set forth in claim 116;

(b) providing a sample comprising polypeptides; and

30 (c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically binds to the polypeptide, thereby isolating or identifying a polypeptide having a xylose isomerase activity.

128. A method of making an anti-xylose isomerase antibody comprising administering to a non-human animal a nucleic acid as set forth in claim 1 or claim 30, or a polypeptide as set forth in claim 75, in an amount sufficient to generate a humoral immune response, thereby making an anti-xylose isomerase antibody.

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129. A method of producing a recombinant polypeptide comprising the
of:

- (a) providing a nucleic acid operably linked to a promoter; wherein the nucleic acid comprises a sequence as set forth in claim 1 or claim 30; and
- (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

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130. The method of claim 130, further comprising transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

131. The method of claim 130, wherein the cell is a plant cell.

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132. A method for identifying a polypeptide having a xylose isomerase activity comprising the following steps:

- (a) providing a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid having a sequence as set forth in claim 1 or claim 30;
- (b) providing a xylose isomerase substrate; and
- (c) contacting the polypeptide or a fragment or variant thereof of step (a) with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a xylose isomerase activity.

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133. The method of claim 132, wherein the substrate is a glucose.

134. A method for identifying a xylose isomerase substrate comprising the following steps:

(a) providing a polypeptide as set forth in claim 76 or a polypeptide encoded by a nucleic acid having a sequence as set forth in claim 1 or claim 30;

(b) providing a test substrate; and

(c) contacting the polypeptide of step (a) with the test substrate of step (b) and
5 detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a xylose isomerase substrate.

135. A method of determining whether a test compound specifically binds
10 to a polypeptide comprising the following steps:

(a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid has a sequence as set forth in claim 1 or claim 30, or, providing a polypeptide as set forth in claim 75;

15 (b) providing a test compound;

(c) contacting the polypeptide with the test compound; and

(d) determining whether the test compound of step (b) specifically binds to the polypeptide.

20 136. A method for identifying a modulator of a xylose isomerase activity comprising the following steps:

(a) providing a polypeptide as set forth in claim 76 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30;

(b) providing a test compound;

25 (c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the xylose isomerase, wherein a change in the xylose isomerase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the xylose isomerase activity.

30 137. The method of claim 136, wherein the xylose isomerase activity is measured by providing a xylose isomerase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product.

138. The method of claim 137, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies
5 the test compound as an activator of xylose isomerase activity.

139. The method of claim 137, wherein an increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies
10 the test compound as an inhibitor of xylose isomerase activity.

140. A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 75, or
15 subsequence thereof, and the nucleic acid comprises a sequence as set forth in claim 1 or claim 30, or subsequence thereof.

141. The computer system of claim 140, further comprising a sequence comparison algorithm and a data storage device having at least one reference sequence stored
20 thereon.

142. The computer system of claim 141, wherein the sequence comparison algorithm comprises a computer program that indicates polymorphisms.

25 143. The computer system of claim 140, further comprising an identifier that identifies one or more features in said sequence.

144. A computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence
30 as set forth in claim 75, or subsequence thereof, and the nucleic acid comprises a sequence as set forth in claim 1 or claim 30, or subsequence thereof.

145. A method for identifying a feature in a sequence comprising the steps of:

(a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 75 or subsequence thereof, and the nucleic acid comprises a sequence as set forth in claim 1 or claim 30 or subsequence thereof; and

(b) identifying one or more features in the sequence with the computer program.

146. A method for comparing a first sequence to a second sequence comprising the steps of:

(a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 75, or subsequence thereof, and the nucleic acid comprises a sequence as set forth in claim 1 or claim 30, or subsequence thereof; and

(b) determining differences between the first sequence and the second sequence with the computer program.

147. The method of claim 146, wherein the step of determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms.

148. The method of claim 146, further comprising an identifier that identifies one or more features in a sequence.

149. The method of claim 146, comprising reading the first sequence using a computer program and identifying one or more features in the sequence.

150. A method for isolating or recovering a nucleic acid encoding a polypeptide with a xylose isomerase activity from an environmental sample comprising the steps of:

(a) providing an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide with a xylose isomerase activity, wherein the primer pair is capable of amplifying SEQ ID NO:1 or SEQ ID NO:3, or a subsequence thereof;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,

(c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a xylose isomerase activity from an environmental sample.

151. The method of claim 150, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, or a subsequence thereof.

152. A method for isolating or recovering a nucleic acid encoding a polypeptide with a xylose isomerase activity from an environmental sample comprising the steps of:

(a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or claim 30, or a subsequence thereof;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);

(c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and

(d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a xylose isomerase activity from an environmental sample.

153. The method of claim 150 or claim 152, wherein the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample.

154. The method of claim 153, wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

155. A method of generating a variant of a nucleic acid encoding a polypeptide with a xylose isomerase activity comprising the steps of:

(a) providing a template nucleic acid comprising a sequence as set forth in claim 1
5 or claim 30; and

(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

156. The method of claim 155, further comprising expressing the variant
10 nucleic acid to generate a variant xylose isomerase polypeptide.

157. The method of claim 155, wherein the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo
15 mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSMTM), synthetic ligation reassembly (SLR) and a combination thereof.

158. The method of claim 155, wherein the modifications, additions or
20 deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial
25 gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

159. The method of claim 155, wherein the modifications, additions or deletions are introduced by error-prone PCR.

160. The method of claim 155, wherein the modifications, additions or deletions are introduced by shuffling.

161. The method of claim 155, wherein the modifications, additions or deletions are introduced by oligonucleotide-directed mutagenesis.

5 162. The method of claim 155, wherein the modifications, additions or deletions are introduced by assembly PCR.

163. The method of claim 155, wherein the modifications, additions or deletions are introduced by sexual PCR mutagenesis.

164. The method of claim 155, wherein the modifications, additions or deletions are introduced by in vivo mutagenesis.

165. The method of claim 155, wherein the modifications, additions or deletions are introduced by cassette mutagenesis.

15 166. The method of claim 155, wherein the modifications, additions or deletions are introduced by recursive ensemble mutagenesis.

20 167. The method of claim 155, wherein the modifications, additions or deletions are introduced by exponential ensemble mutagenesis.

168. The method of claim 155, wherein the modifications, additions or deletions are introduced by site-specific mutagenesis.

25 169. The method of claim 155, wherein the modifications, additions or deletions are introduced by gene reassembly.

170. The method of claim 155, wherein the modifications, additions or deletions are introduced by synthetic ligation reassembly (SLR).

30 171. The method of claim 155, wherein the modifications, additions or deletions are introduced by gene site saturated mutagenesis (GSSM™).

172. The method of claim 155, wherein the method is iteratively repeated until a xylose isomerase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced.

5 173. The method of claim 172, wherein the variant xylose isomerase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature.

10 174. The method of claim 172, wherein the variant xylose isomerase polypeptide has increased glycosylation as compared to the xylose isomerase encoded by a template nucleic acid.

15 175. The method of claim 172, wherein the variant xylose isomerase polypeptide has a xylose isomerase activity under a high temperature, wherein the xylose isomerase encoded by the template nucleic acid is not active under the high temperature.

20 176. The method of claim 155, wherein the method is iteratively repeated until a xylose isomerase coding sequence having an altered codon usage from that of the template nucleic acid is produced.

25 177. The method of claim 155, wherein the method is iteratively repeated until a xylose isomerase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

30 178. A method for modifying codons in a nucleic acid encoding a polypeptide with a xylose isomerase activity to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with a xylose isomerase activity comprising a sequence as set forth in claim 1 or claim 30, or a nucleic acid encoding the polypeptide of claim 75; and,

(b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a

codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

179. A method for modifying codons in a nucleic acid encoding a xylose isomerase polypeptide, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with a xylose isomerase activity comprising a sequence as set forth in claim 1 or claim 30, or a nucleic acid encoding the polypeptide of claim 75; and,

(b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a xylose isomerase.

180. A method for modifying codons in a nucleic acid encoding a xylose isomerase polypeptide to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a xylose isomerase polypeptide comprising a sequence as set forth in claim 1 or claim 30, or a nucleic acid encoding the polypeptide of claim 75; and,

(b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

181. A method for modifying a codon in a nucleic acid encoding a polypeptide having a xylose isomerase activity to decrease its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a xylose isomerase polypeptide comprising a sequence as set forth in claim 1 or claim 30, or a nucleic acid encoding the polypeptide of claim 75; and

(b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding

sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell.

5 182. The method of claim 180 or 181, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

10 183. A method for producing a library of nucleic acids encoding a plurality of modified xylose isomerase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the following steps:

15 (a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, or a subsequence thereof, or, a nucleic acid encoding the polypeptide of claim 75, and the nucleic acid encodes a xylose isomerase active site or a xylose isomerase substrate binding site;

20 (b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

25 (c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified xylose isomerase active sites or substrate binding sites.

 184. The method of claim 183, comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system.

30 185. The method of claim 183, comprising mutagenizing the first nucleic acid of step (a) by a method comprising gene site-saturation mutagenesis (GSSM™).

 186. The method of claim 183, comprising mutagenizing the first nucleic acid of step (a) by a method comprising a synthetic ligation reassembly (SLR).

187. The method of claim 183, further comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM™), synthetic ligation reassembly (SLR) and a combination thereof.

188. The method of claim 183, further comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

189. A method for making a small molecule comprising the following steps:
(a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a xylose isomerase enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 30;
(b) providing a substrate for at least one of the enzymes of step (a); and
(c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.

190. A method for modifying a small molecule comprising the following steps:

(a) providing a xylose isomerase enzyme, wherein the enzyme comprises an amino acid sequence as set forth in claim 75, or, is encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 30;
(b) providing a small molecule; and
(c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the xylose isomerase enzyme, thereby modifying a small molecule by a xylose isomerase enzymatic reaction.

191. The method of claim 190, comprising a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the xylose isomerase enzyme.

192. The method of claim 190, further comprising a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions.

193. The method of claim 190, further comprising the step of testing the library to determine if a particular modified small molecule which exhibits a desired activity is present within the library.

194. The method of claim 193, wherein the step of testing the library further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

195. A method for determining a functional fragment of a xylose isomerase enzyme comprising the steps of:

(a) providing a xylose isomerase enzyme, wherein the enzyme comprises an amino acid sequence as set forth in claim 75, or, is encoded by a nucleic acid having a sequence as set forth in claim 1 or claim 30; and

(b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a xylose isomerase activity, thereby determining a functional fragment of a xylose isomerase enzyme.

196. The method of claim 195, wherein the xylose isomerase activity is measured by providing a xylose isomerase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product.

5 197. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps:

(a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 or claim 30, or a nucleic acid encoding the polypeptide of claim 75;

(b) culturing the modified cell to generate a plurality of modified cells;

(c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and,

15 (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

20 198. The method of claim 197, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene.

25 199. The method of claim 197, further comprising selecting a cell comprising a newly engineered phenotype.

200. The method of claim 199, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

30 201. A method of increasing thermotolerance or thermostability of a xylose isomerase polypeptide, the method comprising glycosylating a xylose isomerase polypeptide, wherein the polypeptide comprises at least thirty contiguous amino acids of a sequence as set forth in claim 75, or a polypeptide encoded by a nucleic acid having a sequence as set forth in claim 1 or claim 30, thereby increasing the thermotolerance or thermostability of the xylose isomerase polypeptide.

202. The method of claim 201, wherein the xylose isomerase specific activity is thermostable or thermotolerant at a temperature in the range from greater than about 90°C to about 130°C.

5

203. A method for overexpressing a recombinant xylose isomerase polypeptide in a cell comprising expressing a vector comprising a nucleic acid comprising a nucleic acid sequence at least 96% sequence identity to the nucleic acid of claim 1 or claim 30 over a region of at least about 100 residues, wherein the sequence identities are
10 determined by analysis with a sequence comparison algorithm or by visual inspection, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

204. A kit comprising a polypeptide as set forth in claim 75 or a
15 polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30, wherein the polypeptide comprises a xylose isomerase activity.

205. A method for catalyzing the isomerization of a glucose to a fructose comprising the following steps:

- 20 (a) providing a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30, wherein the polypeptide comprises a xylose isomerase activity;
- (b) providing a composition comprising a glucose; and
- (c) contacting the polypeptide of step (a) with the glucose of step (b) under
25 conditions wherein the polypeptide of step (a) can isomerase the glucose to a fructose, thereby producing a fructose.

206. A method for producing fructose from a starch comprising the following steps:

- 30 (a) providing a polypeptide capable of hydrolyzing a α -1,4-glycosidic linkage in a starch;
- (b) contacting the polypeptide of the step (a) with the starch under condition wherein the polypeptide of step (a) can hydrolyze α -1,4-glycosidic linkages in the starch, thereby liquefying the starch to produce glucose;

(c) providing a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30, wherein the polypeptide comprises a xylose isomerase activity; and

(d) contacting the polypeptide of step (c) with the glucose of step (b) under conditions wherein the polypeptide of step (c) can isomerase glucose, thereby producing fructose.

207. The method of claim 206, wherein the polypeptide of step (a) comprises an xylose isomerase or a glucoamylase.

208. The method of claim 206 further in step (a) comprising a polypeptide capable of hydrolyzing α -1,6-glycosidic linkage in a starch.

209. A method for producing fructose comprising the following steps:

(a) providing a glucose;

(b) providing a polypeptide having a xylose isomerase activity, wherein the polypeptide comprises an amino acid sequence as set forth in claim 75, or, a polypeptide encoded by a nucleic acid having a sequence as set forth in claim 1 or claim 30; and

(c) contacting the polypeptide of step (b) with the glucose of step (a) under conditions wherein the polypeptide can isomerase glucose thereby producing fructose.

210. The method as set forth in claim 209, wherein the conditions comprise a temperature of between about 70°C and 95°C, thereby shifting equilibrium of the reaction towards formation of fructose.

211. The method as set forth in claim 210, wherein the conditions comprise a temperature of between about 80°C and 90°C, thereby shifting equilibrium of the reaction towards formation of fructose.

212. The method as set forth in claim 209, wherein the polypeptide of step (b) is immobilized.

213. A method of making fructose in a feed or a food prior comprising the following steps:

(a) obtaining a feed or a food material comprising a starch,
(b) providing a polypeptide capable of hydrolyzing a α -1,4- glycosidic linkage in a starch;

(c) contacting the polypeptide of the step (a) with the feed or a food material
5 under conditions wherein the polypeptides of step (a) can hydrolyze α -1,4- glycosidic linkages in the starch to produce a glucose;

(d) providing a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30, wherein the polypeptide comprises a xylose isomerase activity; and

10 (e) adding the polypeptide of step (d) to the feed or food material in an amount sufficient to cause isomerization of the glucose to a fructose in the food or the feed.

214. The method as set forth in claim 213, wherein the food or feed comprises rice, corn, barley, wheat, legumes, or potato.

15 215. The method of claim 206 further in step (a) comprising a polypeptide capable of hydrolyzing α -1,6-glycosidic linkage in a starch.

216. A method for producing a high-fructose syrup comprising the
20 following steps:

(a) providing a polypeptide capable of hydrolyzing α -1,4- glycosidic linkages in a starch;

(b) providing a composition comprising a starch;

(c) contacting the polypeptides of step (a) and the composition of step (b)
25 under conditions wherein the polypeptide of step (a) can hydrolyze α -1,4- glycosidic linkages in the starch;

(d) providing a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30, wherein the polypeptide comprises a xylose isomerase activity; and

30 (e) contacting the polypeptide of step (d) and the starch hydrolysate of step (c) under conditions wherein the polypeptide of step (d) can isomerase glucose in the starch hydrolysate to a fructose, thereby producing the high-fructose syrup.

217. The method of claim 216, wherein the composition comprises a rice, a corn, a barley, a wheat, a legume, a potato or a sweet potato.

218. The method of claim 218, wherein the composition comprises a rice and the high-fructose syrup is a high-fructose corn syrup.

219. The method of claim 216 further in step (a) comprising a polypeptide capable of hydrolyzing α -1,6-glycosidic linkage in a starch.

220. The method of claim 216, wherein all reactions are carried out in one vessel.

221. The method of claim 216, wherein the high-fructose syrup comprises an insecticide bait composition

222. A method for producing a high-fructose syrup comprising the following steps:

(a) providing a transgenic seed or grain comprising a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30, comprising a xylose isomerase activity, wherein the seed or grain comprises a starch;

(b) expressing the xylose isomerase in the seed or grain;

(c) hydrolyzing the starch to a glucose under conditions wherein the polypeptide of step (a) expressed in the seed or grain can catalyze isomerization of glucose to a fructose, thereby producing the high-fructose syrup.

223. The method of claim 221, wherein the steps of hydrolyzing the starch and isomerizing the glucose are carried out at pH 4.0 to 6.5 and at temperature comprising a range of about 55°C to 105°C.

224. A method for producing fructose in brewing or alcohol production comprising the following steps:

(a) providing a polypeptide as set forth in claim 75 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 30, wherein the polypeptide comprises a xylose isomerase activity;

(b) providing malt or mash composition comprising a glucose; and
(c) contacting the polypeptide of step (a) with the composition of step (b)
under conditions wherein the polypeptide of step (a) isomerizes the glucose of step (b) to a
fructose, thereby producing fructose for brewing or alcohol production.

5

225. An isolated or recombinant polypeptide comprising a sequence as set
forth in SEQ ID NO:6.

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226. An isolated or recombinant polypeptide encoded by a nucleic acid
comprising a sequence as set forth in SEQ ID NO:6.

227. An isolated or recombinant nucleic acid comprising a sequence as set
forth in SEQ ID NO:5.

15

228. An isolated or recombinant nucleic acid encoding a polypeptide
comprising a sequence as set forth in SEQ ID NO:6.

229. A double-stranded inhibitory RNA (RNAi) molecule comprising a
subsequence of a sequence as set forth in claim 1 or claim 30.

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230. The double-stranded inhibitory RNA (RNAi) molecule of claim 229,
wherein the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex
nucleotides in length.

25

231. A method of inhibiting the expression of a xylose isomerase in a cell
comprising administering to the cell or expressing in the cell a double-stranded inhibitory
RNA (iRNA), wherein the RNA comprises a subsequence of a sequence as set forth in claim
1 or claim 30.

30

232. An amplification primer pair for amplifying a nucleic acid encoding a
polypeptide having a xylose isomerase activity, wherein the primer pair is capable of
amplifying a nucleic acid comprising a sequence as set forth in claim 1 or claim 30, or a
subsequence thereof.

233. The amplification primer pair of claim 232, wherein a member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence, or, about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more consecutive bases of the sequence.

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234. An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of SEQ ID NO:1; SEQ ID NO:3 or SEQ ID NO:5, and a second member having a sequence as set forth by about the first (the 5') 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of the complementary strand of the first member.

235. A xylose isomerase-encoding nucleic acid generated by amplification of a polynucleotide using an amplification primer pair as set forth in claim 234.

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236. The xylose isomerase-encoding nucleic acid of claim 235, wherein the amplification is by polymerase chain reaction (PCR).

237. The xylose isomerase-encoding nucleic acid of claim 235, wherein the nucleic acid generated by amplification of a gene library.

20

238. The xylose isomerase-encoding nucleic acid of claim 237, wherein the gene library is an environmental library.

239. An isolated or recombinant protease encoded by a xylose isomerase-encoding nucleic acid as set forth in claim 235.

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